

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SCIENCE @ DIRECT®

Biochimica et Biophysica Acta 1660 (2004) 1–6



## Rapid report

## Modulation of KCNQ4 channel activity by changes in cell volume

Charlotte Hougaard<sup>a</sup>, Dan A. Klaerke<sup>b</sup>, Else K. Hoffmann<sup>a</sup>,  
Søren-Peter Olesen<sup>b</sup>, Nanna K. Jorgensen<sup>b,\*</sup><sup>a</sup>Biochemical Department, The August Krogh Institute, University of Copenhagen, Universitetsparken 13, DK-2100 Copenhagen, Denmark<sup>b</sup>Department of Medical Physiology, The Panum Institute, University of Copenhagen, Blegdamsvej 3, 12.520 DK-2200 Copenhagen, Denmark

Received 14 November 2003; accepted 21 November 2003

## Abstract

KCNQ4 channels expressed in HEK 293 cells are sensitive to cell volume changes, being activated by swelling and inhibited by shrinkage, respectively. The KCNQ4 channels contribute significantly to the regulatory volume decrease (RVD) process following cell swelling. Under isoosmotic conditions, the KCNQ4 channel activity is modulated by protein kinases A and C, G protein activation, and a reduction in the intracellular  $\text{Ca}^{2+}$  concentration, but these signalling pathways are not responsible for the increased channel activity during cell swelling.  
© 2003 Elsevier B.V. All rights reserved.

**Keywords:** KCNQ2/3; Cell swelling; PKA; PKC; Tyrosine phosphatase

Most cell types respond to an increase in cell volume by undergoing a regulatory volume decrease (RVD) response, which often is mediated by loss of KCl through separate  $\text{K}^+$  and  $\text{Cl}^-$  channels [1]. Different  $\text{K}^+$  channels, depending on cell type, have been described to play a role in the swelling-induced  $\text{K}^+$  loss. These channels include Kv1.3 and Kv1.5 in lymphocytes, see Ref. [2],  $\text{Ca}^{2+}$ -activated  $\text{K}^+$  channels of small conductance (SK) in, e.g. human liver cells [3], of intermediate conductance (IK) in human T lymphocytes [4] and intestine 407 cells [5] as well as big conductance (BK) channels in, e.g. rabbit kidney proximal tubule cells [6] and human osteoblasts [7]. Activation of SK and IK channels by cell swelling is also described for cloned channels expressed in *Xenopus* oocytes [8] and HEK 293 cells [9]. A TASK channel was suggested as the molecular identity of the volume-sensitive  $\text{K}^+$  conductance in Ehrlich cells [10] and when expressed in HEK 293 cells TASK-2 was found to be sensitive to cell volume changes [11]. Finally, KCNQ1 channels are activated by cell swelling when expressed in COS 7 cells [12] and *Xenopus* oocytes [13]. Swelling-induced activation of KCNQ4 channels was suggested by a study on channels heterologously expressed in *Xenopus* oocytes [13]. In the present study, the sensitivity of KCNQ4 and KCNQ2/3 channels towards changes in cell volume was

further investigated following stable expression in HEK 293 cells. Physiological regulation of KCNQ4 channels is currently poorly described, and we have thus investigated the possible regulatory role of PKA, PKC, tyrosine phosphorylation,  $\text{Ca}^{2+}$  and G proteins under iso- as well as hypoosmotic conditions.

To examine whether KCNQ4 channels are sensitive to changes in cell volume HEK 293 cells stably expressing the channel [14] were exposed to changes in extracellular osmolarity. Since an increase in cell volume will result in the activation of a volume-sensitive  $\text{Cl}^-$  conductance ( $I_{\text{Cl,vol}}$ ) [15], the whole-cell  $\text{K}^+$  current ( $I_{\text{K}}$ ) was followed over time by pulsing to the equilibrium potential for  $\text{Cl}^-$  ( $E_{\text{Cl}}$ ). As seen from Fig. 1A hypoosmotic cell swelling induced a rapid increase in  $I_{\text{K}}$  in KCNQ4 expressing HEK 293 cells, whereas a hyperosmotic challenge completely abolished the current. In some experiments, like the one illustrated in Fig. 1A, inhibition of the KCNQ4 current by the hyperosmotic challenge appeared biphasic. However, this was not the case in other experiments, see e.g. Fig. 2A. The whole-cell  $\text{Cl}^-$  current ( $I_{\text{Cl}}$ ) was followed by pulsing to  $E_{\text{K}}$ . Fig. 1A demonstrates that under the experimental conditions employed in the present study cell swelling activates only a small  $I_{\text{Cl,vol}}$  which likewise was abolished by the hyperosmotic solution. The voltage-dependency of the whole-cell current under iso-, hypo- and hyperosmotic conditions is illustrated in Fig. 1B. The I/V curves shown in Fig. 1C were constructed at the time points indicated by arrows in Fig. 1B.

\* Corresponding author. Tel.: +45-35327347; fax: +45-35327555.  
E-mail address: [nannak@mfi.ku.dk](mailto:nannak@mfi.ku.dk) (N.K. Jorgensen).

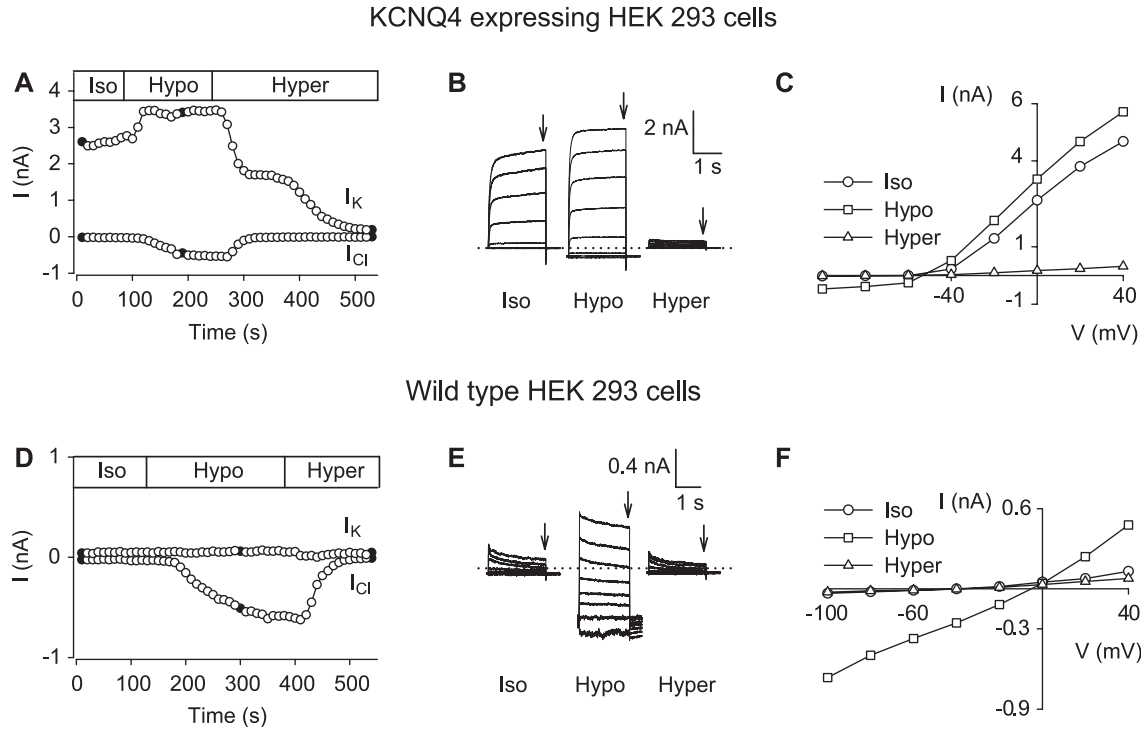


Fig. 1. Effect of osmotic changes on whole-cell  $K^+$  ( $I_K$ ) and  $Cl^-$  ( $I_{Cl}$ ) currents in KCNQ4-expressing and wild type HEK 293 cells. Cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% foetal calf serum and 100 U/ml penicillin–100  $\mu$ g/ml streptomycin at 37 °C and 5%  $CO_2$ . Standard whole-cell patch-clamp experiments were performed at 37 °C essentially as described previously [10,35]. The pipette solution contained (in mM): 2 NaCl, 8 KCl, 108 K gluconate, 1.2  $MgCl_2$ , 10 HEPES, 0.1 EGTA, 1.5  $Na_2ATP$ , pH 7.4 and adjusted to 295 mOsm using D-mannitol. Upon cell swelling, the intracellular  $K^+$  and  $Cl^-$  concentration is calculated to be diluted to 96 and 10 mM, respectively [36]. The extracellular hypoosmotic (200 mOsm) solution was (in mM): 1 NaCl, 5 KCl, 90 Na gluconate, 1  $MgCl_2$ , 1  $CaCl_2$ , 10 HEPES, pH 7.4. Isoosmotic (300 mOsm) and hyperosmotic (400 mOsm) solutions were made by addition of D-mannitol. The holding potential was  $-80$  mV, and the following voltage protocol was applied every 10 s: a 1 s step to  $-78$  mV ( $E_K$ ), a 1 s step to  $-80$  mV followed by a 2 s step to 0 mV ( $E_{Cl}$ ).  $I_{Cl}$  was measured at a small window at  $E_K$  and  $I_K$  at the end of the 2 s pulse to  $E_{Cl}$ . (A) Time course of  $I_K$  and  $I_{Cl}$  in KCNQ4-transfected HEK 293 cells following changes in extracellular osmolarity. (B) Voltage-dependent properties of the whole-cell current in KCNQ4-transfected cells. From the holding potential of  $-80$  mV, 20 mV steps of 2 s duration was applied from  $-100$  to  $40$  mV under iso-, hypo- and hyperosmotic conditions at the time points indicated with filled symbols in Panel A. (C) Current/voltage ( $I/V$ ) relationship calculated from the current traces shown in B at the time points indicated by the arrows. (D) Time-course of the whole-cell currents in wild type (non-transfected) HEK 293 cells. All experimental conditions were as in A, but please note the change in Y-axis. (E) Voltage-dependent properties of the whole-cell current in non-transfected cells. All experimental conditions were as in B. (F)  $I/V$  relationship calculated from the current traces shown in E at the time points indicated by the arrows. The figure is representative of 16 KCNQ4-expressing cells and four untransfected cell experiments, respectively. Mean KCNQ4 currents under iso- and hypoosmotic conditions are depicted in Fig. 4.

Under isoosmotic conditions where virtually no  $Cl^-$  current was present, stepping the potential from  $-100$  to  $+40$  mV resulted in a slowly activating current characteristic of KCNQ4 [14]. Hypoosmotic cell swelling activated KCNQ4 channels, inducing an increase in current at all potentials, whereas hyperosmotic cell shrinkage strongly inhibited the KCNQ4 current (Fig. 1B). The reversal potential of the current under hypoosmotic conditions is slightly shifted towards more positive potentials, indicating contribution from  $I_{Cl,vol}$  as well as KCNQ4 channels. Fig. 1D–F show results from similar experiments performed on untransfected HEK 293 cells. These experiments confirm that KCNQ4 channels mediate the swelling-induced increase in  $I_K$  shown in Fig. 1A–C, as no increase in  $I_K$  is observed under hypoosmotic conditions in wild-type HEK 293 cells. The  $I_{Cl,vol}$  observed is similar to that reported previously [15].

The isoosmotic as well as the swelling-induced  $I_K$  in KCNQ4 expressing HEK 293 cells was completely

inhibited by the KCNQ channel inhibitors linopirdine (50  $\mu$ M,  $n=4$ , not illustrated) and bepridil (20  $\mu$ M,  $n=3$ , not illustrated), corroborating that the swelling-induced  $I_K$  is KCNQ4 mediated. It should be noted that bepridil besides inhibiting KCNQ4 also blocked  $I_{Cl,vol}$  in these cells (not shown). The effect of bepridil on  $I_{Cl,vol}$  has not been further investigated. The sensitivity of KCNQ4 channels to cell volume changes observed in the present study is in agreement with the data previously published for channels expressed in *Xenopus* oocytes [13].

Retigabine, an anticonvulsant, activates KCNQ-type channels including KCNQ4 [16,17] and KCNQ2/3 [18]. To investigate whether hypoosmotic exposure induced maximal activation of the KCNQ4 channels, retigabine was added both under iso- and hypoosmotic conditions (Fig. 2A). As expected, retigabine induced a reversible increase in  $I_K$  under isoosmotic conditions. A hypoosmotic challenge induced an increase in the KCNQ4 current amplitude, which

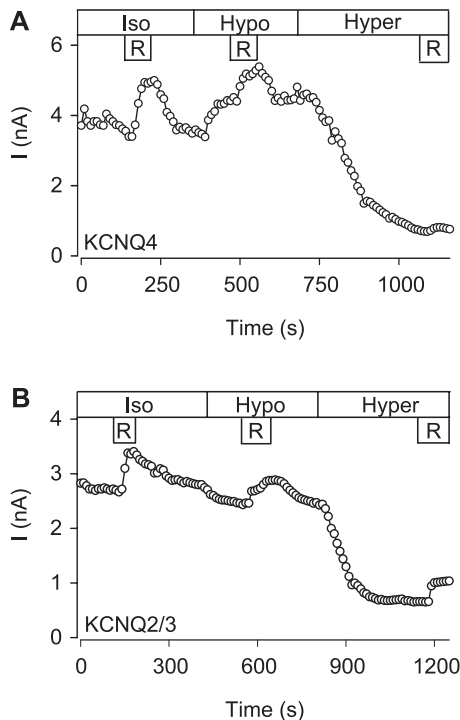


Fig. 2. Effect of the KCNQ channel opener retigabine on the KCNQ4 (A) and KCNQ2/3 (B) currents under different osmotic conditions. The whole-cell  $I_K$  was measured as described in the legend to Fig. 1. Cells were exposed to isoosmotic (300 mOsm), hypoosmotic (200 mOsm), or hyperosmotic (400 mOsm) extracellular solutions, and retigabine (10  $\mu$ M) as indicated. (A,  $n=3$ ; B,  $n=4$ ).

was further stimulated by application of retigabine, suggesting that the degree of cell swelling induced by the present experimental conditions did not cause maximal activation of the KCNQ4 channels. During hyperosmotic conditions, where strong inhibition of KCNQ4 channels was observed, application of retigabine induced only a small increase in the KCNQ4 current level (Fig. 2A). Whether this reflects that the channels are strongly inhibited by the hyperosmotic challenge or perhaps even removed from the membrane following prolonged cell shrinkage is unclear. It should be noted that while short-term exposure ( $<2$  min) to a hyperosmotic challenge induces a reversible inhibition of the channels, prolonged application of the hyperosmotic solution leads to an irreversible inhibition.

To examine whether the closely related KCNQ2/3 heteromeric channel complex is sensitive to cell volume changes, HEK 293 cells expressing these channels [17] were exposed to changes in extracellular osmolarity as illustrated in Fig. 2B. Unlike the KCNQ4 current, KCNQ2/3 currents were not potentiated by a hypoosmotic challenge, but these channels were still inhibited by hyperosmotic exposure. Prolonged exposure to hypoosmotic solutions (5 min) likewise failed to activate the KCNQ2/3 channels ( $n=3$ , not illustrated). Application of retigabine increased  $I_K$  under all osmotic conditions (Fig. 2B), confirming the presence of functional KCNQ2/3 channels and that the lack of activation

by cell swelling is probably not due to maximal activation of the current under isoosmotic conditions. Following expression in *Xenopus* oocytes, KCNQ2/3 channel activity was not sensitive neither to cell swelling nor to cell shrinkage [13]. One possible explanation for this apparent discrepancy could be differences in the degree of cell shrinkage between *Xenopus* oocytes and mammalian cells, another could be other differences in expression system and thereby in possible signalling pathways involved in channel inhibition during osmotic cell shrinkage.

The observation that KCNQ4 channels are activated by cell swelling in whole-cell patch-clamp experiments does not per se demonstrate that these channels are involved in the RVD process. To verify that KCNQ4 channels are activated by cell swelling in intact cells and contribute to volume recovery, cell volume changes following changes in extracellular osmolarity were measured using large angle light scattering (Fig. 3). As seen from Fig. 3A, exposing the cells to a hypoosmotic solution resulted in fast cell swelling followed by a period of RVD. Upon return to isoosmotic

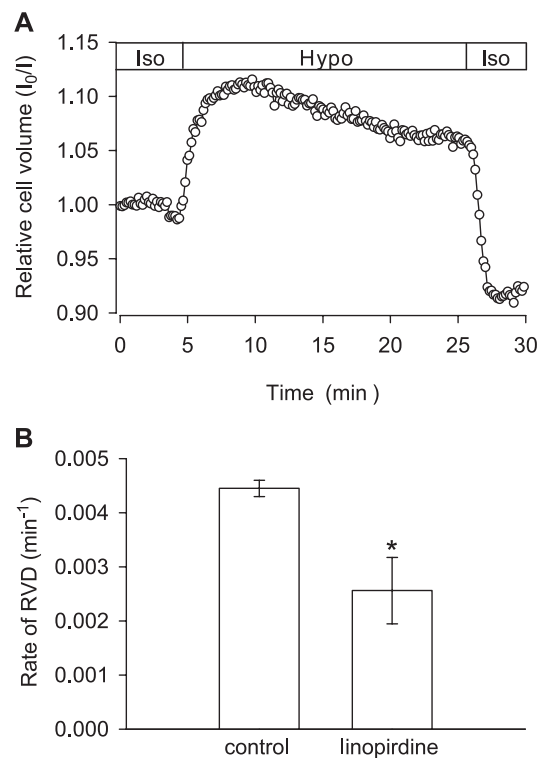


Fig. 3. KCNQ4-expressing HEK 293 cells exhibit linopirdine-sensitive RVD. (A) Cell volume changes as a function of time. Relative cell volume was measured using large-angle light scattering as described in Ref. [9]. The isoosmotic solution (300 mOsm) contained (in mM): 133 NaCl, 5 KCl, 1  $\text{CaCl}_2$ , 1  $\text{Na}_2\text{HPO}_4$ , 1  $\text{MgSO}_4$ , 0.5  $\text{NaH}_2\text{PO}_4$ , 10 HEPES, 5 glucose, pH 7.4. In the hypoosmotic solution (200 mOsm), NaCl was reduced to 85 mM. (B) Effect of linopirdine on the RVD rate in KCNQ4-expressing HEK 293 cells. RVD rates were calculated as the slope of the relative cell volume traces in the initial 300 s following maximal cell swelling in the absence (control,  $n=5$ ) or presence of 100  $\mu$ M linopirdine ( $n=4$ ) in the hypoosmotic solution. (\*) Indicates statistic different from control ( $P<0.05$ , Student's  $t$ -test for unpaired observations).

medium cell volume decreased below the initial value demonstrating that osmotic active substances have been lost from the cells. To verify the contribution of KCNQ4 channels to RVD, cells were exposed to the hypoosmotic solution in the absence (control) or presence of the KCNQ channel blocker, linopirdine (Fig. 3B). As seen, linopirdine significantly inhibited the RVD rate in KCNQ4-expressing HEK 293 cells, demonstrating that in the intact cell system, KCNQ4 channels are activated by cell swelling and contribute to the RVD process. The residual RVD observed in the presence of linopirdine could perhaps be due to KCl loss via a KCl co-transport mechanism, which is unlikely to be affected by this channel blocker.

Modulation of KCNQ4 channels by cellular signalling pathways is not well described. Here we investigated the possible role of classical pathways such as PKC, PKA, tyrosine phosphorylation, G proteins and  $\text{Ca}^{2+}$  as modulators of KCNQ4 activity both under isoosmotic and hypo-

osmotic conditions (Fig. 4). Interference with these pathways has prominent effects on RVD and osmolyte loss following swelling in numerous cell types, for review see, e.g. Refs. [19,20]. Activation of PKC by short-term exposure to PMA significantly inhibited the KCNQ4 current under isoosmotic conditions, but was without effect on the potentiation of the current by cell swelling (Fig. 4A). Activation of PKA by 8-Br-cAMP stimulated, whereas inhibition of PKA by Rp-cAMP was without effect on the isoosmotic KCNQ4 current, demonstrating that PKA activation stimulates KCNQ4 channels but is not required for normal KCNQ4 activity (Fig. 4B). Neither stimulation nor inhibition of PKA affected the hypoosmotic potentiation of the KCNQ4 current (Fig. 4B). In agreement with these observations, PKC was found to inhibit and PKA to stimulate the activity of the closely related KCNQ1 channel under isoosmotic conditions but to be without effect on the activation of these channel by increases in cell volume [13].

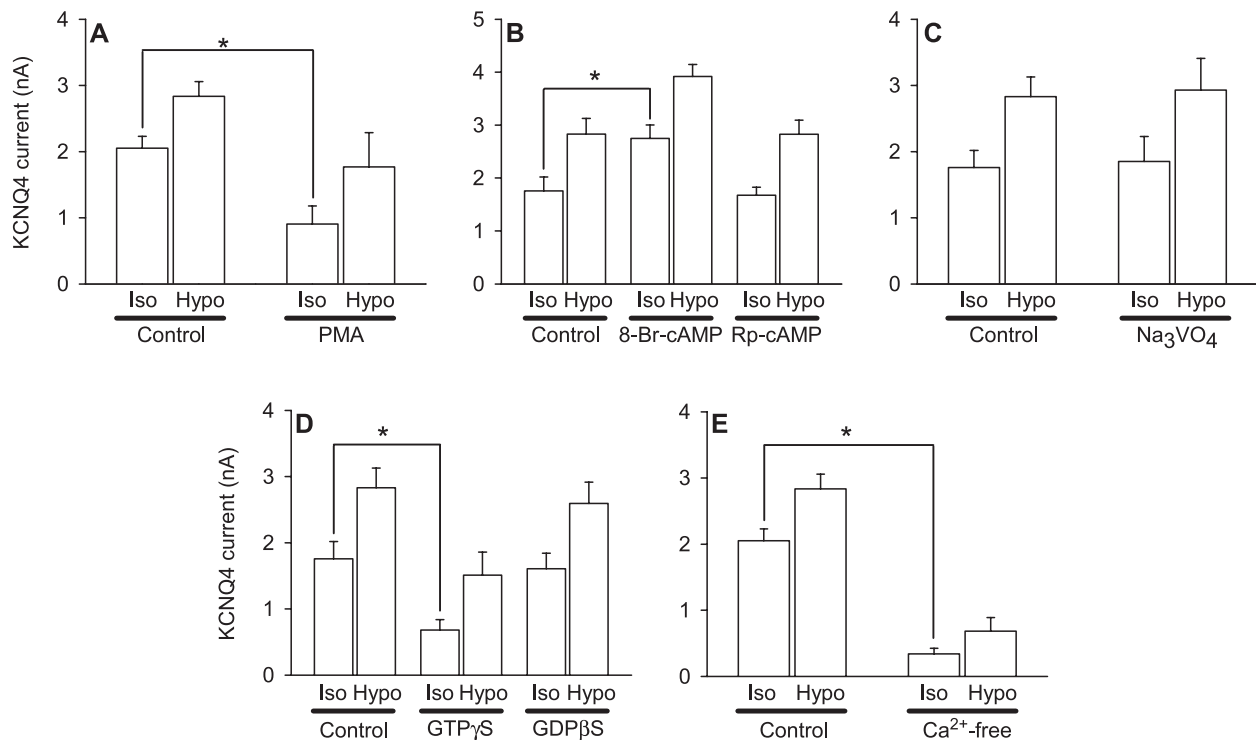


Fig. 4. Modulation of the KCNQ4 current by cellular signalling pathways. In all experiments, the KCNQ4 current magnitude was measured at 0 mV under isoosmotic conditions (Iso) and 90 s after hypoosmotic cell swelling (Hypo). (A) The role of PKC in modulation of KCNQ4 activity. PMA (100 nM) was added to the perfusion solution after obtaining a stable current level under isoosmotic conditions ( $n=6$ ). Control experiments ( $n=16$ ) were performed in a similar manner but in the absence of PMA. (B) PKA as a modulator of KCNQ4 channels. 8-Br-cAMP (1 mM,  $n=9$ ) or Rp-cAMP (1 mM,  $n=5$ ) was added to the pipette solution. Experiments were initiated 8 min after establishing the whole-cell configuration to allow adequate equilibration between the pipette solution and the intracellular compartment. Control experiments ( $n=10$ ) were performed in a similar manner but in the absence of 8-Br-cAMP and Rp-cAMP. (C) Role of tyrosine phosphorylation in modulation of KCNQ4 channels. Experiments were conducted as in B, but  $\text{Na}_3\text{VO}_4$  (400  $\mu\text{M}$ ,  $n=5$ ) was added to the pipette solution. (D) Role of G-proteins in the modulation of KCNQ4 channels. Experiments were conducted as in B except that GTP $\gamma$ S (40  $\mu\text{M}$ ,  $n=7$ ) or GDP $\beta$ S (1 mM,  $n=6$ ) was added to the pipette solution. (E) Role of  $\text{Ca}^{2+}$  in the modulation of KCNQ4 channels. Experiments were performed at a "permissive"  $\text{Ca}^{2+}$  level (0.1 mM EGTA,  $n=16$ , Control) or in the absence of  $\text{Ca}^{2+}$  (10 mM EGTA,  $n=7$ ). (\*) Indicates significant difference ( $P<0.05$ , Student's  $t$ -test for unpaired experiments). Please note that the current during hypoosmotic exposure in all experiments was significantly higher than under isoosmotic conditions ( $P<0.05$ , paired observations). The swelling-induced increase in current was not significantly different in treated and untreated cells, except in experiments performed in the absence of  $\text{Ca}^{2+}$  ( $P<0.05$ , unpaired experiments). However, in the absence of  $\text{Ca}^{2+}$ , the relative swelling-induced increase was not reduced (please see text for details).



It has been suggested that one of the early signalling events following cell swelling is the activation of a tyrosine kinase. RVD is blunted by inhibitors of tyrosine kinases, see Ref. [21] and protein tyrosine phosphorylation is involved in the regulation of, e.g. the volume-sensitive  $\text{Cl}^-$  channels [22,23], the volume-sensitive taurine efflux pathway [24,25] and the KCl co-transporter [26]. The Src tyrosine kinase decreased the KCNQ4 current amplitude when it was overexpressed together with the channels in Chinese hamster ovary cells [27], suggesting that a tyrosine kinase(s) could play a central role in the regulation of KCNQ4 channel activity. Increasing cellular tyrosine phosphorylation by application of the tyrosine phosphatase inhibitor  $\text{Na}_3\text{VO}_4$  increased the amplitude of the volume-sensitive  $\text{Cl}^-$  current in the HEK 293 cells ( $n=5$ , not shown), similar to previous reports from other cell lines [22,23] but was without effect on both the isoosmotic and the hypoosmotic KCNQ4 current (Fig. 4C). In addition, the specific src kinase inhibitor PP2 (400 nM,  $n=3$ ) had no significant effect on the isoosmotic KCNQ4 current and likewise failed to affect the potentiation of the current by cell swelling. The lack of effect of PP2 on the KCNQ4 current found in the present study might be explained by low endogenous src-like activity in HEK 293 cells. However, taken together, the results obtained in the present study argue against a significant role of tyrosine phosphorylation events in the modulation of KCNQ4 channels by changes in cell volume. Both monomeric and heterotrimeric G proteins seem to be involved in the RVD response, and addition of  $\text{GTP}\gamma\text{S}$ , a compound known to activate G proteins, has been shown to accelerate the RVD response [28,29] and to activate a  $\text{Cl}^-$  conductance with similar properties to the one activated by cell swelling [23]. Activation of G proteins by  $\text{GTP}\gamma\text{S}$  resulted in a reduction in the KCNQ4 current amplitude under isoosmotic conditions (Fig. 4D), but failed to affect the potentiation of the current by cell swelling. The inhibitory effect of  $\text{GTP}\gamma\text{S}$  under isoosmotic conditions is in agreement with several reports demonstrating that M-currents are inhibited following activation of G protein coupled receptors like, e.g. muscarinic, adrenergic and purinergic receptors, see Ref. [30]. In a recent report,  $\text{GTP}\gamma\text{S}$  was found to stimulate the KCNQ4-mediated current when the channels were expressed in *Xenopus* oocytes [31]. The reason for this apparent discrepancy is unclear, but might be explained by differences in the experimental conditions; in this context *Xenopus* oocytes are rather promiscuous with respect to G protein coupling and signal transduction pathways. Inhibition of G proteins by  $\text{GDP}\beta\text{S}$  was without effect on both iso- and hypoosmotic KCNQ4 current activity (Fig. 4D) and taken together the results point against an important role for G proteins in the swelling-induced activation of KCNQ4 channels. It should be noted that  $\text{GTP}\gamma\text{S}$  produced a transient increase in the  $\text{Cl}^-$  current in HEK 293 cells under isoosmotic conditions (not shown) in agreement with previous reports in CPAE cells [23]. Finally, we investigated whether there was a requirement for  $\text{Ca}^{2+}$  in

the activation of the KCNQ4 channels by omitting  $\text{Ca}^{2+}$  from the extracellular solution and buffering intracellular  $\text{Ca}^{2+}$  by high concentrations of EGTA (Fig. 4E). Removing  $\text{Ca}^{2+}$  strongly suppressed the KCNQ4 current under both iso- and hypoosmotic conditions. However, even in the absence of  $\text{Ca}^{2+}$ , cell swelling still produced a significant increase in the KCNQ4 current amplitude demonstrating that  $\text{Ca}^{2+}$  is not the primary messenger responsible for activation of these channels after swelling. At permissive  $\text{Ca}^{2+}$  concentrations the KCNQ4 current during cell swelling was  $1.40 \pm 0.03$  ( $n=16$ ) relative to isoosmotic control, in the absence of  $\text{Ca}^{2+}$  the swelling-induced KCNQ4 current was  $1.90 \pm 0.16$  ( $n=7$ ) relative to isoosmotic control. The role of  $\text{Ca}^{2+}$  in regulation of native M-current activity seems somewhat obscure ranging from inhibiting [32], no role [33] to stimulation [34]. From our experiments, it is clear that a certain  $\text{Ca}^{2+}$  level is required for normal KCNQ4 activity following expression in HEK 293 cells. It is, however, not clear whether the requirement for  $\text{Ca}^{2+}$  is directly related to the channel, or due to effects on intracellular signalling pathways.

In conclusion, we find that KCNQ4 channels expressed in HEK 293 cells are sensitive to cell volume changes, being stimulated by cell swelling and inhibited by cell shrinkage, respectively. Unlike the KCNQ4 channels, KCNQ2/3 heteromeric channels are not activated by cell swelling, but this channel complex can still be inhibited by cell shrinkage. Besides being activated by cell swelling in patch-clamp experiments, KCNQ4 channels also contribute to the RVD process in intact cells. Under isoosmotic conditions PKA, PKC, G proteins and  $\text{Ca}^{2+}$  can modulate the KCNQ4 channels whereas protein tyrosine phosphorylation only plays a minor role in KCNQ4 channel modulation. The potentiation of the KCNQ4 current by cell swelling is unaffected by PKA, PKC, G proteins and protein tyrosine phosphorylation. A certain  $\text{Ca}^{2+}$  level may be required for full KCNQ4 activity after swelling but  $\text{Ca}^{2+}$  is not the primary messenger responsible for the increase in KCNQ4 current induced by cell swelling.

## Acknowledgements

This work was supported by the Carlsberg Foundation (0204/20; 1116/20), The Velux Foundation and The John and Birthe Meyer Foundation.

## References

- [1] E.K. Hoffmann, J.W. Mills, Membrane events involved in volume regulation, *Current Topics in Membranes*, Academic Press, USA, 1999, pp. 123–195.
- [2] R.S. Lewis, M.D. Cahalan, Potassium and calcium channels in lymphocytes, *Annu. Rev. Immunol.* 13 (1995) 623–653.
- [3] R. Roman, A.P. Feranchak, M. Troetsch, J.C. Dunkelberg, G. Kilic, T. Schaack, J. Schaack, J.G. Fitz, Molecular characterization of volume-

- sensitive SK<sub>Ca</sub> channels in human liver cell lines, *Am. J. Physiol.* 282 (2002) G116–G122.
- [4] R. Khanna, M.C. Chang, W.J. Joiner, L.K. Kaczmarek, L.C. Schlichter, hSK4/hIK1, a calmodulin-binding K<sub>Ca</sub> channel in human T lymphocytes. Roles in proliferation and volume regulation, *J. Biol. Chem.* 274 (1999) 14838–14849.
  - [5] J. Wang, S. Morishima, Y. Okada, IK channels are involved in the regulatory volume decrease in human epithelial cells, *Am. J. Physiol.* 284 (2003) C77–C84.
  - [6] L. Dubé, L. Parent, R. Sauvé, Hypotonic shock activates a maxi K<sup>+</sup> channel in primary cultured proximal tubule cells, *Am. J. Physiol.* 259 (1990) F348–F356.
  - [7] M. Weskamp, W. Seidl, S. Grissmer, Characterization of the increase in [Ca<sup>2+</sup>]<sub>i</sub> during hypotonic shock and the involvement of Ca<sup>2+</sup>-activated K<sup>+</sup> channels in the regulatory volume decrease in human osteoblast-like cells, *J. Membr. Biol.* 178 (2000) 11–20.
  - [8] M. Grunnet, N. MacAulay, N.K. Jorgensen, S. Jensen, S.P. Olesen, D.A. Klaerke, Regulation of cloned, Ca<sup>2+</sup>-activated K<sup>+</sup> channels by cell volume changes, *Pflügers Arch.* 444 (2002) 167–177.
  - [9] N.K. Jorgensen, S.F. Pedersen, H.B. Rasmussen, M. Grunnet, D.A. Klaerke, S.P. Olesen, Cell swelling activates cloned Ca<sup>2+</sup>-activated K<sup>+</sup> channels: a role for the F-actin cytoskeleton, *Biochim. Biophys. Acta* 1615 (2003) 115–125.
  - [10] C. Hougaard, F. Jørgensen, E.K. Hoffmann, Modulation of the volume-sensitive K<sup>+</sup> current in Ehrlich ascites tumour cells by pH, *Pflügers Arch.* 442 (2001) 622–633.
  - [11] M.I. Niemeyer, L.P. Cid, L.F. Barros, F.V. Sepúlveda, Modulation of the two-pore domain acid-sensitive K<sup>+</sup> channel TASK-2 (KCNK5) by changes in cell volume, *J. Biol. Chem.* 276 (2001) 43166–43174.
  - [12] T. Kubota, M. Horie, M. Takano, H. Yoshida, H. Otani, S. Sasayama, Role of KCNQ1 in the cell swelling-induced enhancement of the slowly activating delayed rectifier K<sup>+</sup> current, *Jpn. J. Physiol.* 52 (2002) 31–39.
  - [13] M. Grunnet, T. Jespersen, N. MacAulay, N.K. Jorgensen, N. Schmitt, O. Pongs, S.P. Olesen, D.A. Klaerke, KCNQ1 channels sense small changes in cell volume, *J. Physiol.* 549 (2003) 419–427.
  - [14] R. Søgaard, T. Ljungstrøm, K.A. Pedersen, S.P. Olesen, B.S. Jensen, KCNQ4 channels expressed in mammalian cells: functional characteristics and pharmacology, *Am. J. Physiol.* 280 (2001) C859–C866.
  - [15] B. Nilius, J. Prenen, U. Wissenbach, M. Bodding, G. Droogmans, Differential activation of the volume-sensitive cation channel TRP12 (OTRPC4) and volume-regulated anion currents in HEK-293 cells, *Pflügers Arch.* 443 (2001) 227–233.
  - [16] L. Tatulian, P. Delmas, F.C. Abogadie, D.A. Brown, Activation of expressed KCNQ potassium currents and native neuronal M-type potassium currents by the anti-convulsant drug retigabine, *J. Neurosci.* 21 (2001) 5535–5545.
  - [17] R.L. Schröder, T. Jespersen, P. Christophersen, D. Strøbæk, B.S. Olesen, S.P. Olesen, KCNQ4 channel activation by BMS-204352 and retigabine, *Neuropharmacology* 40 (2001) 888–898.
  - [18] L. Tatulian, D.A. Brown, Effect of the KCNQ potassium channel opener retigabine on single KCNQ2/3 channels expressed in CHO cells, *J. Physiol.* 549 (2003) 57–63.
  - [19] F. Lang, G.L. Busch, M. Ritter, H. Volkl, S. Waldegger, E. Gulbins, D. Häussinger, Functional significance of cell volume regulatory mechanisms, *Physiol. Rev.* 78 (1998) 247–306.
  - [20] H. Pasantes-Morales, V. Cardin, K. Tuz, Signaling events during swelling and regulatory volume decrease, *Neurochem. Res.* 25 (2000) 1301–1314.
  - [21] E.K. Hoffmann, Intracellular signalling involved in volume regulatory decrease, *Cell. Physiol. Biochem.* 10 (2000) 273–288.
  - [22] B.C. Tilly, N. van den Berghe, L.G. Tertoolen, M.J. Edixhoven, H.R. de Jonge, Protein tyrosine phosphorylation is involved in osmoregulation of ionic conductances, *J. Biol. Chem.* 268 (1993) 19919–19922.
  - [23] T. Voets, V. Manolopoulos, J. Eggermont, C. Ellory, G. Droogmans, B. Nilius, Regulation of a swelling-activated chloride current in bovine endothelium by protein tyrosine phosphorylation and G proteins, *J. Physiol.* 506 (1998) 341–352.
  - [24] A.A. Mongin, J.M. Reddi, C. Charniga, H.K. Kimelberg, [3H]taurine and D-[3H]aspartate release from astrocyte cultures are differently regulated by tyrosine kinases, *Am. J. Physiol.* 276 (1999) C1226–C1230.
  - [25] I.H. Lambert, B. Falktoft, Lysophosphatidylcholine induces taurine release from HeLa cells, *J. Membr. Biol.* 176 (2000) 175–185.
  - [26] P.W. Flatman, N.C. Adragna, P.K. Lauf, Role of protein kinases in regulating sheep erythrocyte K-Cl cotransport, *Am. J. Physiol.* 271 (1996) C255–C263.
  - [27] N. Gamper, J.D. Stockand, M.S. Shapiro, Subunit-specific modulation of KCNQ potassium channels by Src tyrosine kinase, *J. Neurosci.* 23 (2003) 84–95.
  - [28] C. Lo, J. Ferrier, H.C. Tenenbaum, C.A. McCulloch, Regulation of cell volume and intracellular pH in hyposmotically swollen rat osteosarcoma cells, *Biochem. Cell. Biol.* 73 (1995) 535–544.
  - [29] M.R. Shen, C.Y. Chou, M.L. Wu, K.E. Huang, Differential osmosensing signalling pathways and G-protein involvement in human cervical cells with different tumour potential, *Cell. Signal.* 10 (1998) 113–120.
  - [30] B.S. Brown, S.P. Yu, Modulation and genetic identification of the M channel, *Prog. Biophys. Mol. Biol.* 73 (2000) 135–166.
  - [31] T. Ljungstrøm, M. Grunnet, B.S. Jensen, S.P. Olesen, Functional coupling between heterologously expressed dopamine D<sub>2</sub> receptors and KCNQ channels, *Pflügers Arch.* 446 (2003) 684–694.
  - [32] A.A. Selyanko, D.A. Brown, Intracellular calcium directly inhibits potassium M channels in excised membrane patches from rat sympathetic neurons, *Neuron* 16 (1996) 151–162.
  - [33] P.R. Adams, D.A. Brown, A. Constanti, Pharmacological inhibition of the M-current, *J. Physiol.* 332 (1982) 223–262.
  - [34] S.P. Yu, D.M. O'Malley, P.R. Adams, Regulation of M current by intracellular calcium in bullfrog sympathetic ganglion neurons, *J. Neurosci.* 14 (1994) 3487–3499.
  - [35] C. Hougaard, M.I. Niemeyer, E.K. Hoffmann, F.V. Sepúlveda, K<sup>+</sup> currents activated by leukotriene D<sub>4</sub> or osmotic swelling in Ehrlich ascites tumour cells, *Pflügers Arch.* 440 (2000) 283–294.
  - [36] G. Riquelme, F.V. Sepúlveda, F. Jørgensen, S. Pedersen, E.K. Hoffmann, Swelling-activated potassium currents of Ehrlich ascites tumour cells, *Biochim. Biophys. Acta* 1371 (1998) 101–106.